

TABLE 4-continued

Properties of Proteins				
Protein	Abr.	Molecular Mass	Isoelectric Point	Electrophoretic Mobility in TG buffer ( $\text{cm}^2/\text{V} \cdot \text{s}$ ) $\times 10^{-5}$
Bovine Serum Albumin	BSA	68,000	4.9, 4.7	28.9
Cytochrome c	CYT	12,500	10.2	—

## Results

The net charge and electrophoretic velocity of proteins was determined by the pH of the buffer used. When the buffer TG with a pH of 8.3 was used all of the above proteins were negatively charged with the exception of cytochrome c. These proteins would be expected to migrate towards the positive electrode. On a gellan gum electrophoresis gel the considerable electroosmotic flow carries the proteins toward the negative electrode. Negatively charged proteins with the lowest electrophoretic mobility will migrate toward the negative electrode. Negatively charged proteins with higher electrophoretic mobility (oppose electroosmotic flow to a greater extent) migrate towards the negative electrode at a lower rate. Since gellan gum is negatively charged, positively charged proteins can adsorb to the gel and are slowed in their migration toward the negative electrode. Adding additional polymers to the gel can reduce the electroosmotic flow so that negatively charged proteins will migrate towards the positive electrode. FIG. 5 shows the electrophoresis of proteins in an 0.2% gellan gum electrophoresis gel containing 0.2% polyethylene oxide 5,000,000. The buffer used was TG. Electrophoresis was conducted at 2 V/cm for 20 min and 8.6 V/cm for 184 min at 20° C. The samples were: lane 1, myoglobin; lane 2, cytochrome c; lane 3, bovine serum albumin; lane 4,  $\alpha$ -lactalbumin; lane 5, bovine whey mixture; lane 6,  $\beta$ -lactoglobulin; lane 7,  $\beta$ -lactoglobulin A; lane 8,  $\beta$ -lactoglobulin B. As seen in FIG. 5, the protein with the highest electrophoretic mobility (bovine serum albumin) moved the greatest distance towards the positive electrode. This gel shows the partial resolution of  $\beta$ -lactoglobulin A and B which differ, in electrophoretic mobility by about 8.5%. FIG. 5 also shows that cytochrome c is adsorbed to the gel and does not migrate significantly towards the negative electrode.

These results with proteins indicate that a number of variables can be modified to achieve selective separations. The pH appears to directly determine the charge on the protein and the electrophoretic mobility. Proteins with greater positive charge will adsorb to the negatively charged gel and will be slowed in their migration. The ionic strength of the solution influences adsorption to the gel polymer. The amount of electroosmotic flow in the gel can be controlled and a protein's net migration determined by the magnitude of the electroosmotic flow and the electrophoretic mobility. Additional interactions between the proteins and additional polymers will also determine the separation.

## EXAMPLE 5

### Formation of Gellan Electrophoresis Gels Based on Disulfide Bonds

Proteins are examples of polymers that can be cross-linked through reversible disulfide bonds. Cysteine is an amino acid containing a free sulphydryl (thiol) side chain.

Cysteine can be readily oxidized to for cystine, which contains two cysteines linked at the side chains through a disulfide bond. This is the method nature has evolved to reversibly cross-link proteins. The methyl esters of cysteine and cystine have blocked carboxyl groups and free amino groups. Strong stable gels were formed when cystine dimethyl ester (0.005 mol/L) in 0.01 mol/L TRIS buffer pH 7.0 was used to form a gellan electrophoresis gel (0.1%). Cysteine methyl ester (0.01 mol/L) did not form gels under the same conditions. A solution of dithiothreitol (0.05 mol/L, final concentration) when added to a gellan electrophoresis gel (0.01%) formed with cystine dimethyl ester converted the gel back to solution. These experiments show that gellan electrophoresis gels can be made using disulfide bonds and returned to solution using reducing solutions.

Although the above-described gellan electrophoresis gel uses a diamine (cystine dimethyl ester) to form a gel, thiol groups can also be introduced into the gellan gum polymer by covalent bonds. Gellan gum has a charged carboxyl group that binds cations. The carboxyl groups can also be used as an attachment point to make various gellan gum derivatives. The carboxyl group is a reactive site to which can be covalently attached thiol or other functional groups. The carboxyl group reacts with other groups such as amines when used with compounds such as carbodimides. Carbodimides will promote the condensation of an amine and a carboxyl group. A derivative of gellan gum containing free sulfide groups covalently attached to the carboxydrate chain can be made by such organic chemistry. For instance, if gellan gum is reacted with a compound such as 2-mercaptoethylamine and a carbodiimide. The carboxyl group and amine form an amide. Other reactions can be used to introduce covalently bound sulphydryl groups to form disulfides. Such a gel has the advantage of having no charge and free sulphydryl groups to form reversible gels based on the redox of the solution.

## EXAMPLE 6

### Method to Remove Gellan From Isolated Samples

A solution of  $\text{CaCl}_2$  is added to a sample containing a target molecule, such as DNA or a protein, removed from a gellan gum electrophoresis medium cross-linked with  $\text{CaCl}_2$  to a concentration of 5 mmol/L or greater. The solution is mixed and the cross-linked gellan gum is removed by centrifugation, for example at 12,000 $\times$ g for 15 min, or by filtration. The gellan gum is collapsed into a compact pellet (centrifugation) or retained on a filter (filtration) leaving the target molecule in solution. This method improves the transformation efficiency by DNA in chemically competent cells

Although the present invention has been fully described in conjunction with the preferred embodiment thereof with reference to the accompanying drawings, it is to be understood that various changes and modifications may be apparent to those skilled in the art. Such changes and modifications are to be understood as included within the scope of the present invention as defined by the appended claims, unless they depart therefrom.

What is claimed is:

1. An electrophoresis apparatus comprising a gel comprising gellan gum, a cross-linking agent comprising a divalent metal cation, and a size-separation modifying polymer; and

means for exposing said electrophoresis medium to an electric field.